bio-protocol Preprint

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Histology

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Updated date: Nov 16, 2021

An abbreviated version of this protocol was published in Science Translational Medicine in Jan 2020

Long-gap peripheral nerve repair through sustained release of a neurotrophic factor in nonhuman primates

DOI: 10.1126/scitranslmed.aav7753

Detailed protocol

Histology slide preparation

The distal nerves were sectioned at 5 mm thickness and were adhered onto gelatin coated slides (SupplyMyLab PO101) and left to dry at room temperature for 48 hours. After drying, the slides were placed in a 60^{o}C oven for approximately one hour to melt the paraffin, and then, were subjected to deparaffinization by xylene solution. After deparaffinization, the slides were rehydrated through graded alcohol washes and placed in running tap water, before being submerged in 3% hydrogen peroxide-deionized water solution for 5 minutes. After the peroxide treatment, the slides were placed in an antigen retrieval buffer consisting of sodium citrate at pH 4, and were microwaved (Magic Chef MCD770RW 770 Watts) at 50% power for 25 minutes. After the antigen retrieval, the slides were allowed to cool at room temperature for 10 minutes and were placed in a phosphate-buffered saline solution containing 0.1% Triton-X 100 (Sigma-Aldrich X100-100ML) for 10 minutes for membrane permeabilization. Then, the slides were blocked for non-specific binding by 20% goat serum (Sigma-Aldrich G9023-10ML) solution in 0.1% Triton-X 100- PBS for 45 minutes. After blocking, a 1:50 (v/v) solution containing the Neurofilament primary (Thermo-Fisher Scientific 13-1300) or the S100 primary (Abcam ab14849) and 0.3% bovine-serum albumin in phosphate-buffered saline solution, respectively, were added onto the slides and incubated overnight at 4^{o}C. The next day, slides were washed three times in 0.5% bovine-serum albumin- phosphate-buffered saline solution and blocked again using the 20% goat serum solution for 45 minutes before adding the secondary antibody (Neurofilament NEFH/NEFM/NEFL secondary (Thermo-Fisher Scientific A28175) or the S100 secondary (Abcam ab150115) antibody). The slides were washed in PBS three times post-incubation and two drops of DAPI (Thermo-Fisher Scientific R37606) were added on the slides for 5 minutes before a quick PBS rinse and cover-slipping with Fluoromount (Thermo-Fisher Scientific 00-4958-02).

Masson's Trichrome stain

Masson's Trichrome was used to visualize the degree of collagen deposition within the explanted nerves (Abcam ab150686). Briefly, sectioned (5 mm) slides were deparaffinized and placed in preheated Bouin's solution for 60 minutes. The slides were then cooled for 10 minutes and rinsed in deionized water prior to incubation in Weigert's Iron Hematoxylin (Abcam ab150686) for 5 minutes. After rinsing again, the slides were incubated in Biebrich Scarlet Acid Fuchsin (Abcam ab150686) for 15 minutes. After another short rinse, the slides were differentiated in phosphomolybdic/phosphotungstic acid solution for 15 minutes before being incubated in Aniline Blue for 15 minutes. Slides were incubated in acetic acid for 4 minutes before being dehydrated with graded alcohol washes, mounted using Permount and cover-slipped.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Fadia, N. and Fadia, N. (2021). Histology. Bio-protocol Preprint. bio-protocol.org/prep1443.
2. Fadia, N. B., Bliley, J. M., DiBernardo, G. A., Crammond, D. J., Schilling, B. K., Sivak, W. N., Spiess, A. M., Washington, K. M., Waldner, M., Liao, H., James, I. B., Minteer, D. M., Tompkins-Rhoades, C., Cottrill, A. R., Kim, D., Schweizer, R., Bourne, D. A., Panagis, G. E., II, M. A. S., Egro, F. M., Campwala, I. K., Simpson, T., Weber, D. J., II, T. G., Brooker, J. E., Josyula, T., Guevara, A. A., Repko, A. J., Mahoney, C. M. and Marra, K. G.(2020). Long-gap peripheral nerve repair through sustained release of a neurotrophic factor in nonhuman primates . Science Translational Medicine 12(527). DOI: 10.1126/scitranslmed.aav7753